***In vitro* autoradiographic localization of [³H]neuropeptide Y-binding sites in rat brain**

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ABSTRACT

The location of [³H]neuropeptide Y (NPY)-binding sites in rat brain was investigated with *in vitro* autoradiographic approach and image analysis by IBAS-2. The predominant densities of [³H]NPY-binding sites were found in superficial layers of the cortex, septal nucleus, multiple thalamic nuclei and hippocampal formation. Ammon's horn had the highest densities among the hippocampal subfields. In the hippocampal region, [³H]NPY-binding sites were distributed in a laminar fashion, with higher densities in stratum radiatum followed by those in stratum oriens. Stratum pyramidale, lacunosum and moleculare were poor in the specific binding sites. The results suggest that NPY may play an important role in the limbic system.

Neuropeptide Y (NPY), originally isolated from the extract of porcine brain, contains 36 amino acid residues and belongs to the pancreatic polypeptide (PP) family of regulatory peptides (29). NPY is widely distributed in both the central and peripheral nervous systems (1, 3, 21, 27). In mammalian brain, NPY occurs in higher concentrations than any other known peptides (1, 3), suggesting an important role of this peptide in brain function. NPY is also known to work together with catecholamine in brain as well as in the periphery, where a portion of NPY-containing neurons represents classical sympathetic neurons (21, 27). In order to better understand the actions of NPY, the clarification of its specific receptors seems to be important. We have visualized the distribution of [³H]NPY-binding sites in slices of rat brain by use of *in vitro* autoradiography and image analysis, and obtained somewhat different results from those reported previously (10, 23).

Wistar rats weighing approximately 200 g were decapitated, and the brains were rapidly removed and cut into 10- μ m thick sections in a cryostat. The sections were mounted on

gelatin-coated glass slides and stored at -70°C until use. The brain sections were preincubated five times for 3 min each in HEPES (*N*-2-hydroxy-ethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (19), and then incubated for 30 min at room temperature in the same buffer containing 0.1 mM bacitracin, 0.5% bovine serum albumin and 2 nM of [³H]NPY (specific activity, 68.3 Ci/mmol; Amersham). Nonspecific binding was assessed by adding 10 μ M of unlabelled NPY (Peninsula Laboratories) to the incubation buffer. Sections were washed five times for 2 min each in HEPES buffer, dipped in distilled water for 20 sec, and dried in a stream of cold air. The slides were tightly juxtaposed against tritium-sensitive films (³H-Ultrofilm; LKB) and exposed for 6 weeks at -70°C . The autoradiograms were analyzed by an IBAS-2 image analyzer (Zeiss).

As shown in Fig. 1 and Fig. 2A, the highest densities of [³H]NPY-binding sites were observed in 1) superficial layers of the cortex, 2) lateral septal nucleus, 3) various thalamic nuclei including anteromedial, anteroventral, mediodorsal, and medial geniculate nucleus, and 4) hippocampus. Other regions had rela-

tively poor densities. Nonspecific binding throughout the brain was low. In the hippocampal formation (Fig. 2B), a heterogeneous pattern of labelling was observed among various subfields in each section. Ammon's horn had the highest densities of [3 H]NPY-binding sites, and moderate densities were also observed in dentate gyrus. However, subiculum, presubiculum, parasubiculum, and entorhinal area were obviously poor in [3 H]NPY-binding sites. Within Ammon's horn, [3 H]NPY-binding sites were distributed in a laminar fashion with the highest densities seen in stratum radiatum followed by stratum oriens. Other layers, the alveus, stratum pyramidale and stratum lacunosum-moleculare were relatively poor in the specific binding sites.

The abundance of NPY-like immunoreactivity in various regions of the mammalian central nervous system, such as the cortex, thalamus, septum, amygdala and hippocampus (1, 3, 17), implies the multifunction of this peptide. Recent studies have indicated that NPY exerts influence upon the central cardiovascular regulation (5, 12, 26), hypothalamic-pituitary-adrenal axis (13, 16, 31), memory process (9), sexual behavior (7), feeding (7, 20, 25), circadian rhythm (2) and thermoregulation (14, 24). For these actions, the specific receptors appeared to have an important role, which were studied by membrane assay (15, 30) or by *in vitro* autoradiography (10, 19, 22, 23, 26).

Our present results, which revealed high concentrations of NPY-binding sites in superficial cortex, hippocampus, lateral septum and thalamus, are in general agreement with previous reports (10, 19, 22, 23). In this study, however, specific binding sites in olfactory bulb and substantia nigra were not so plentiful, although Giardino *et al.* (10) and Martel *et al.* (23) have reported the high densities of 125 I-Bolton-Hunter NPY receptor sites in these regions. The differences were also observed in the thalamus, for example the higher densities of anterior nuclei and medial geniculate body as shown in this report. These inconsistencies may be, in part, related to the methodological differences, including the radioligands used.

Of particular interest is the fact that hippocampus has higher densities of NPY-binding sites, without exception, in various mam-

Fig. 1 Autoradiogram from horizontal rat brain section after specific binding with 2 nM of [3 H]NPY. Nonspecific binding sections with 10 μ M unlabelled NPY added are indistinguishable from the background (not shown).

lian species including humans, and the heterogeneous distribution is seen among hippocampal subfields (10, 19, 22, 23). In the present study, the highest concentration of NPY-binding sites was observed in the stratum radiatum of Ammon's horn; Köhler *et al.* have recently reported the highest densities of specifically bound [3 H]NPY in the stratum radiatum of the regio inferior (19). Stratum oriens also had higher densities, but other layers of Ammon's horn did not. Such a laminated pattern of NPY receptor location in the dendritic zone of pyramidal cells is in agreement with previous reports and is likely to be a common aspect in rodents (19, 22). These results suggest that NPY affects the hippocampal functions controlled through local interneurons and other extrinsic projections.

It is well-known that hippocampus has varied neuronal populations and extrinsic and intrinsic projections, and that NPY coexists with other neuroactive substances (6, 18). Recent studies with rats have demonstrated that NPY acts presynaptically in area CA1 of the hippocampus to reduce excitatory input to

Fig. 2 Color-coded transforms of autoradiograms from horizontal rat brain sections. Relative binding densities are indicated by the color spectrum shown in A and B (hippocampal region), such that red corresponds to the highest binding and blue to the lowest. These approximate bregma coordinates are +5.5. AM, anteromedial thalamic nucleus; AV, anteroventral thalamic nucleus; CA, cornu ammonis; COR, cortex; DG, dentate gyrus; EA, entorhinal area; H, hippocampus; LS, lateral septal nucleus; MD, mediodorsal thalamic nucleus; MG, medial geniculate nucleus; PaS, parasubiculum; PrS, presubiculum; S, subiculum; sl-m, stratum lacunosum-moleculare; so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum

the pyramidal neurons (8, 11), and that NPY has a direct excitatory action on granule cells in the dentate gyrus (4). NPY is reported to inhibit forskolin- or vasoactive intestinal peptide-stimulated adenylate cyclase activity in membranes from rat (28) or dog (unpublished data) hippocampus. We have also demonstrated that NPY receptors are proteins having a molecular weight of 50,000, without subunits connected by sulphhydryl bonds (15). Therefore, NPY appears to have much influence on hippocampal functions via its receptors linked to adenylate cyclase and other second messenger systems.

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